to probe the functions of any locally expressed protein provided that it is turned over at an appreciable rate compared to the duration of the experiment. The ability to inhibit protein synthesis efficiently in a spatially discrete manner should open the way for a myriad of studies exploring the role of protein patterning in intra- and intercellular signaling.

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Off the Shelf but Not Mass Produced

Using high-throughput screening, Jo et al. in this issue of *Chemistry & Biology* [1] have identified SEW2871 as a structurally unique sphingosine 1-phosphate$_1$ (S1P$_1$) receptor agonist. SEW2871 binds to and activates the S1P$_1$ receptor and initiates a survival signaling pathway similar to that of S1P.

The lysolipid sphingosine is a ubiquitous component of cells and cell membranes. One or more isoforms of the enzyme sphingosine kinase is known to phosphorylate sphingosine. The resultant molecule, sphingosine 1-phosphate (S1P), acts as a ligand to activate a family of heptahelical membrane-spanning receptors. Activated S1P receptors, of which there are five subtypes (S1P$_{1-5}$), bind to guanine nucleotide-containing proteins (G proteins) that are, in turn, coupled to downstream signaling pathways involved in the regulation of vascular development, cell survival, proliferation, and motility. S1P also regulates lymphocyte egress from lymph nodes and the thymus gland, thereby influencing immune function. When lymphocyte egress is blocked, a decrease in circulating lymphocytes (lymphopenia) ensues, and the ability of the organism to mount an immune response is impaired. Thus, S1P or analogs mimicking its function could be useful in reducing the immune response, leading to organ rejection in patients receiving liver, kidney, heart, or other transplants.

In this issue of *Chemistry & Biology*, Jo et al. [1] report on the use of high-throughput screening of commercial chemical libraries to identify an agonist of the S1P$_1$ receptor. Becuase the agonist, named SEW2971, was already present among the compounds screened and did not require either chemical design or synthesis, they have used the term “off the shelf” to describe this molecule. Using S1P$_1$ receptor modeling and mutagenesis studies, the authors found that despite major structural dissimilarities between S1P and SEW2971, headgroup binding S1P$_1$ receptor residues are required for kinase activation by both compounds through a combination of hydrophobic and ion-dipole interactions. SEW2871 actions both resemble and are different from the S1P receptor agonist FTY720, an immunosuppressant drug currently in phase III clinical trials in patients receiving kidney transplants. FTY720 not only produces lymphopenia but also downregulates and degrades S1P receptors [2]. In contrast, S1P and SEW2871 both induce lymphopenia but allow S1P receptor recycling after downregulation [1, 3, 4]. Such recycling may preserve other biological effects of the S1P receptor(s) involved. Another difference is that FTY720 acts on three different S1P receptor subtypes (S1P$_{1,2,5}$) [3], whereas the modeling studies of Jo et al. [1] indicate high selectivity of SEW2871 for the S1P$_1$ receptor subtype. The latter observations will require confirmation by physiologic and biochemical studies.

Both S1P and SEW2871 activate survival signals, including Rac GTPase, ERK1/2, and Akt, through a G protein-coupled (Gi/o) mechanism [1]. SEW2871 was considerably less potent in activating these signals compared with S1P or with AFD(R), a phosphate ester of the chiral FTY analog AAL-(R) [1]. Whether this reduced potency means that larger doses will be required to achieve effective systemic concentrations must await future studies.

In addition to the immune system, sphingolipids also exert substantial influence on the cardiovascular system [5]. For example, reduction of blood supply by obstructed coronary blood vessels leading to tissue

ischemia results in myocardial infarction, i.e., heart attack. Restoration of blood flow (reperfusion) causes tissue injury via mitochondrial damage and free radical generation. In the isolated mouse heart, tissue survival after acute ischemia/reperfusion injury is enhanced by S1P and by ganglioside GM-1, an activator of sphingosine kinase [6]. The benefit of ischemic preconditioning, in which the heart is subjected to brief periods of ischemia/reperfusion before prolonged ischemia/reperfusion injury, relies on activation of sphingosine kinase, which is ϵPKC dependent [7]. Survival signals activated by S1P identified so far are similar to those described above and include the PI-3 kinase/PKB (Akt), ERK, and JNK pathways [8]. In mouse heart, these pathways require intact $S1P_2$ and $S1P_3$ receptors [8]. Thus, it will be of interest to test the effects of SEW2871 on cell survival signals in which receptor subtypes other than $S1P_1$ have been identified by genetic deletion or biochemical inhibitor studies.

If SEW7921 activates the signaling pathways described above, cell survival should ensue. As indicated above, no data for SEW7921 are available in heart cells or tissue, nor are there any studies in liver or brain. Similarly lacking are inhibitor studies of the PI-3 kinase and the arms of the MAP kinase pathways that would further define the signaling results reported by Jo et al. [1]. Particularly illuminating would be studies involving the PKC pathway, especially ϵPKC, which has been implicated in protection against ischemic and hypoxic damage in the heart [6, 9] and other tissues. Use of ϵPKC-null mice [6] would help to define the role of this isozyme in the SEW7921 pathway both in the heart and in T lymphocytes where $S1P_1$ receptor recycling induced by the naturally occurring ligand S1P is ϵPKC dependent [3]. All of these studies would constitute the next logical steps in learning more about the signaling pathways activated by SEW7921.

FTY720 attenuates hepatic ischemia/reperfusion injury in rats with either normal or cirrhotic livers [10]. Tissue survival was associated with activation of the Akt pathway but with downregulation of the MAP kinase pathway. This is in contrast to SEW271, in which stimulation of the MAP kinase pathway was observed [1]. How such activation affects cell survival requires additional study and may be cell and tissue specific. Also, it will be necessary to determine whether new S1P-aryl-amide-containing compounds that are antagonists of $S1P_1$ and $S1P_3$ receptors [11] will block stimulation by SEW7921.

Another important topic is to learn how blood vessels supplying vital organs respond to agonists that activate S1P receptors. Control of these responses is achieved in part by release of nitric oxide (NO) from vascular endothelial cells. NO causes relaxation of coronary arteries and, thereby, increases blood flow to ischemic myocardium. NO is produced in vascular endothelial cells via activation of endothelial nitric oxide synthase or eNOS, and S1P stimulates activation of eNOS [12]. However, S1P given acutely has also been reported to constrict blood vessels in some species [5]. In this regard, FTY720 and SEW2971 will require comparison with S1P, both with respect to acute and chronic hemodynamic effects and NO release.

Sphingolipids are also important in cancer cell biology. In some malignant cells, including leukemia cells, hepatoma and lymphoma cell lines, and human glioma cells, FTY720 does not promote cell survival but rather induces apoptosis by several mechanisms, such as phosphatase activation and dephosphorylation of Akt pathway factors, mitochondrial cytochrome C release, and caspase-6 activation [13–16]. Whether SEW2971 will have similar dual effects as FTY720 in normal versus malignant cells remains to be determined. Some of these effects involve mitochondria, but no data regarding mitochondrial function, such as cytochrome C release, permeability-transition-pore opening, or effects on electron-transport-chain function, are available for SEW2971. Similarly, its toxicity profile has yet to be studied.

In summary, Jo et al. have demonstrated that an "off-the-shelf," nondesigner drug can mimic a naturally occurring, physiologically relevant molecule. Although it is "off the shelf," SEW2971 has not been mass produced. SEW2971 or its successors may evolve into a class of "boutique" drugs with highly specialized uses. The elegant molecular studies described in this paper will be valuable across a broad range of disciplines and represent the development of a new tool with which to study a variety of pathophysiological processes.

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